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(54) Title: IRREVERSIBLE PEPTIDE LIGANDS FOR BOMBESIN RECEPTORS

(57) Abstract

Peptides of formula A-B-C-D-Gln-Trp-Ala-Val-X-Y-T-W (either A = H, Boc; AC; one of B and C = pMel, mMel; the other of B and C = valence bond, Gly, Leu-Gly, E-Leu-Gly, Gln-E-Leu-Gly,E,E-Gly with E = Arg(A), arg(A), Lys(A), lys(A), Orn(A), orn(A) or A = H, B = Glp-Arg-Leu-Gly, C = pMel or mMel; and D = valence bond, Asn, Thr; X = Gly, ala; Y = valence bond, His(R_1), his(R_1), Phe, phe, Ser, ser, Ala, ala; T = valence bond, Leu, leu, Phe, phe; W = OH, $NH_2NH(CH_2)_4CH_3$, $NH(CH_2)_2C_6H_5$, Met-R_2, Leu-R_2, Ile-R_2, Nle-R_2; R_1 = H, Tos, Dnp, Bzl; R_2 = NH_2, OH, OCH_3, $NHNH_2$) and their pharmaceutically acceptbale salts are irreversible peptide ligands for Bombesin receptors. Their preparation and pharmaceutical compositions containing them are also described.

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IRREVERSIBLE PEPTIDE LIGANDS FOR BOMBESIN RECEPTORS

Description

The present invention relates to new biologically active peptides, their pharmaceutically acceptable salts, and the processes for their preparation and application as therapeutic agents.

In this specification symbols and abbreviations are those commonly used in peptide chemistry (see Eur.J. Biochem. (1984) 138, 9-37). Consequently, the three-letter amino acid symbols denote the L configuration of chiral amino acids. D-amino acids are represented by small letters: e.g., ala = D-Ala. Other symbols and abbreviations used are: AA, amino acid; AcOEt, ethylacetate; AcOH, acetic acid; Bzl, benzyl; BBS, bombesin; Boc, t-butyloxycarbonyl; BuOH, butyl alcohol; CCD, counter-current distribution; DCC, N,N'-dicyclohexylcarbodiimide; dec., decomposition; DMAP, 4-dimethylaminopyridine; DMF, dimethylformamide; Dnp, 2,4-dinitrophenyl; ECC, ethylchlorocarbonate; Et_2O, diethylether; Glp, L-pyroglutamic acid; h-GRP (or p-GRP), human (or porcine) gastrin releasing peptide; HCl/AcOH, dry HCl in anhydrous acetic acid; HOBt, 1-hydroxybenzotriazole; i.c.v., intracerebroventricular; MeOH, methyl alcohol; m.p. melting point; mMel= m-bis(2-chloroethyl)amino-L-phenylalanine; n.d., not determined; NMM, N-methylmorpholine; pMel= p-bis (2-chloroethyl) amino-L-phenylalanine; HPLC, high performance liquid chromatography;

OSu, N-hydroxysuccinimidyl; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin layer chromatography; Tos, p-toluensulphonyl; TsOH, p-toluensulphonic acid; Z, benzyloxycarbonyl.

More particularly, the present invention relates to peptides having bombesin antagonistic activity useful in the therapy of human neoplasms which are depending from peptides of the GRP family.

Other bombesin antagonists have been prepared in the past, but those peptides, however, showed moderate affinity for the BBS receptors. (A. Cowan (1988) TIPS, 9,1-3);

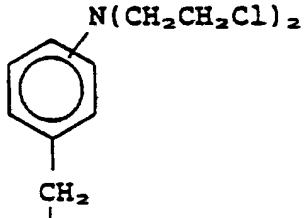
The invention provides peptides of formula (I)

A—B—C—D—Gln—Trp—Ala—Val—X—Y—T—W (I)

1 2 3 4 5 6 7 8 9 10 11 12

where:

A= H,Boc,Ac



B= pMel,mMel (-Mel-=-HN-CH-CO-)

C= -(valence bond),Gly,Leu-Gly, E -Leu-Gly,Gln- E -Leu-Gly,E,E-Gly

D= -,Asn,Thr

E= Arg(A), arg(A), Lys(A), lys(A), Orn(A), orn(A)

X= Gly,ala

Y= -,His(R_1),his(R_1),Phe,phe,Ser,ser,Ala,ala

T= -,Leu,leu,Phe,phe

W= OH,NH_2,$NH(CH_2)_4CH_3$,$NH(CH_2)_2C_6H_5$,Met-R_2, Leu-R_2,Ile-R_2,Nle-R_2

R_1= H,Tos,Dnp,Bzl

R_2= NH_2,OH,OMe,NH-NH_2

B and C can be inverted (B in 3 and C in 2); in this case, when A= H,Gln-Arg-Leu-Gly may become Glp-Arg-Leu-Gly.

Salts of these peptides with pharmaceutically acceptable acids are within the scope of the invention. Such acid addition salts can be derived from a variety of inorganic and organic acids such as sulfuric, phosphoric, hydrochloric, hydrobromic, hydroiodic, nitric, sulfamic, citric, lactic, pyruvic, oxalic, maleic, succinic, tartaric, cinnamic, acetic, trifluoracetic, benzoic, salicylic, gluconic, ascorbic and related acids.

Bombesin (BBS) is a tetradecapeptide of formula Glp-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2, originally isolated from the skin of a frog. The biological activity resides in the C-terminal part of the molecule. BBS(6-14)nonapeptide is as active as the parent compound. The human counterpart of bombesin is a 27 amino acid peptide known as gastrin-releasing peptide (h-GRP). Bombesin and bombesin-like peptides display a number of biological activities (J.H. Walsh (1983) in "Brain Peptides", D.T. Krieger, M.J. Brownstein and J.B. Martin (eds), Wiley Interscience Publ., pp. 941-960), including autocrine growth-promoting effects on human small cell lung carcinoma (SCLC) (F. Cuttitta et al. (1985) Cancer Survey, 4, 707-727), autocrine and/or paracrine stimulation of human prostatic cancer cell proliferation (M. Bologna et al., Cancer, in press) and modulation of the EGF receptor (I. Zachary and E. Rozengurt (1985) Cancer Surveys, 4, 729-765).

In this case, a bombesin antagonist, by competing with the natural ligand for the receptor(s), would inhibit or modify the triggering of the cascade of events leading to abnormal cell proliferation.

The alkylating bombesin analogues of the formula I are bombesin receptor antagonists and can, therefore, find application in the therapy of human neoplasm which are modulated in their growth and progression by peptides of the GRP family, either directly or in concert with other growth factors.
In addition, these alkylating analogues can be used in the management of the clinical symptoms associated with these deseases and due to hypersecretion of GRP-like peptides.

The compounds of the invention can be administered by the usual routes, for example, parenterally, e.g. by intravenous injection or infusion, or by intramuscular, subcutaneous, intracavity and intranasal administration.
The dosage depends on the age, weight and condition of the patient and on the administration route.
On the basis of the "in vitro" and "in vivo" data in mice it can be estimated that the therapeutic doses in humans will be in the range 10 ng/kg - 10 mg/kg, once to 6 times daily.
The invention also provides
pharmaceutical compositions containing a compound of formula (I) as the active substance, in association with one or more pharmaceutically acceptable excipients.
The pharmaceutical compositions of the invention are usually prepared following conventional methods and are administered in a pharmaceutically suitable form.
For instance, solutions for intravenous injection or infusion may contain as carrier, for example, sterile water or, preferably, they may be in the form of sterile aqueous isotonic saline solutions.
Suspensions or solutions for intramuscular injections may contain, together with the active compound, a pharmaceutically acceptable carrier, e.g. sterile water, olive oil, ethyl oleate, glycols, e.g. propylene glycol, and, if desired, a suitable amout of lidocaine hydrochloride.
Furthermore, according to the invention there is provided a method of treating neuroendocrine neoplasms, such as small cell

lung carcinoma and prostatic carcinoma or the clinical symptoms associated with these diseases in a patient in need of it, comprising administering to the said patient a composition of the invention.

Chemistry

The synthesis of the peptides of the invention may be accomplished by classical solution methods. The synthesis consists essentially of appropriate successive condensations of protected amino acids or peptides. The condensations are carried out so that the resulting peptides have the desired sequence of amino acid residues.

The amino acids and peptides, which can be condensed according to methods known in peptide chemistry, have the amino and carboxyl groups, not involved in peptide bond formation, blocked by suitable protecting groups capable of being removed by acid or alkali treatment or by hydrogenolysis.

For the protection of the amino group the following protective groups may, for example, be employed: benzyloxycarbonyl, t-butoxycarbonyl, trityl, formyl, trifluoracetyl, o-nitrophenylsulphenyl, 4-methyloxybenzyloxycarbonyl, 9-fluorenylmethoxycarbonyl, 3,5-dimethoxy-α-α'-dimethylbenzyloxycarbonyl or methylsulphonylethoxycarbonyl.

For the protection of the carboxyl group the following protective groups may, for example, be employed: methyl, ethyl, t-butyl, benzyl, p-nitrobenzyl or fluorenylmethyl, amide, hydrazide, t-butoxycarbonyl hydrazide or benzyloxycarbonyl hydrazide.

The hydroxy functions of hydroxy amino acids and the imino function of histidine may be protected by suitable protecting groups (throughout all the synthesis or only during a few steps) or may be unprotected. For the protection of the hydroxy function the following protective groups may, for example, be employed; t-butyl,

benzyl, acetyl. For the protection of the imidazole imino function the following groups may, for example, be used: 2,4-dinitrophenyl, tosyl, benzyl. De-protecting reactions are carried out according to methods known per se in peptide chemistry.

The condensation between an amino group of one molecule and a carboxyl group of another molecule to form the peptidic linkage may be carried out through an activated acyl-derivative such as a mixed anhydride, an azide or an activated ester, or by direct condensation between a free amino group and a free carboxyl group, in the presence of a condensing agent such as dicyclohexylcarbodiimide, alone or together with a racemization preventing agent, such as N-hydroxysuccinimide or 1-hydroxybenzotriazole, or together with an activating agent such as 4-dimethylamino-pyridine. The condensation may be carried out in a solvent such as dimethylformamide, dimethylacetamide, pyridine, acetonitrile, tetrahydrofuran or N-methyl-2-pyrrolidone.

The reaction temperature may be from -30°C to room temperature. The reaction time is generally from 1 to 120 hours.

The scheme of synthesis, the protecting groups and condensing agents are selected so as to avoid the risk of racemization.

R_f values are determined on pre-coated plates of silica gel 60 F_{254} (Merck), layer thickness 0.25 mm, length 20 cm, using the following development systems:

System A: ethyl acetate/benzene/acetic acid/water
= 500/500/100/50 by volume (upper phase)

System B: ethyl acetate/benzene/acetic acid/water = 500/500/200/75 by volume (upper phase)

System C: n-butanol/acetic acid/water = 600/150/150 by volume

System D: chloroform/methanol/NH_4OH 30% = 488/338 150 by volume

System E: chloroform/methanol = 90/10 by volume

System F: toluene/ethylacetate/acetic acid/water = 100/100/20/10 by volume

TLC analyses are carried out at a temperature ranging from 18°C to 25°C: the R_f values can therefore change ± 5%.

High performance liquid chromatography (HPLC) was carried out using a Hewlett-Packard 1084B apparatus equipped with a UV detector operating at 210 nm. The peptides are separated on a 4 x 250 mm Lichrosorb RP 18 5µ column. The following solvents are used:

A) 0.02 M KH_2PO_4 adjusted to pH 3.5 with 3% H_3PO_4/CH_3CN= 9/1 by volume

B) 0.02 M KH_2PO_4 adjusted to pH 3.5 with 3% H_3PO_4/CH_3CN= 3/7 by volume.

The elution is programmed with a linear gradient from 60% to 90% B over a period of 20 min (System A) or from 30 to 70% B over a period of 15 min (System B), and then isocratically for 15 min, with a flow rate of 1 ml/min.

The peptides are characterized by their retention time (RT).

Amino acid analysis have been carried out on acid hydrolysates (either at 110°C for 22 h in 6 N HCl + 0.1% phenol or at 100°C for 16 h in 3 N mercaptoethansulfonic acid, both under N_2). Only

natural amino acid residues have been determined. Due to partial decomposition in normal hydrolysis conditions, Trp has been determined only in hydrolysates with the sulfonic acid.

Biology

The binding affinity of the compounds of the present invention for the bombesin receptors has been determined on mouse Swiss 3T3 fibroblasts (I. Zachary and E. Rozengurt (1985) Proc. Natl. Acad. Sci. USA, 82, 7616-7620) (Table 1).

The effect on mitogenesis has been determined in quiescent and confluent Swiss 3T3 cells maintained in serum free medium (A.N.Corps et al (1985) Biochem J. 231, 781-785). In a first set of experiments, analogues are given alone or in combination with bombesin. In a second set of experiments, cells are pre-treated with the alkylating peptides, washed, left at 37°C for 24 hours and then challenged with bombesin. In both cases, DNA synthesis has been evaluated as [H^3]thymidine incorporation (Table 2).

Mitogenic effect of bombesin and its analogues have been also evaluated as activation of the protein-tyrosin kinase that phosphorylates a 115 KD protein (p115) associated with the bombesin receptor complex (D.Cirillo et al. (1986) Mol.Cell. Biol. 6, 4641-4649) (Table 3).

In addition, exposure to these peptides in the 0.1-50 µM range was associated with significant reduction in the growth of SCLC cell lines (such as NCI-H345, NCI-N592, NCI-H128), as well as of prostatic carcinoma cell lines (such as DU145 and PC3).

Parenteral administration of these peptides at doses ranging between 10 ng/kg - 10 mg/kg to nude mice was associated with significant growth reduction of the above mentioned transplanted human SCLC and prostatic carcinoma cell lines.

Peripheral and central effects have been evaluated in the rat, respectively "in vitro", as urinary bladder contraction (M. Broccardo et al. (1975) Br. J. Pharmac., 55, 221-227) and "in vivo" by i.c.v. administration, as grooming behaviour (A.Cowan et al. (1985) Life Sciences, 37, 135 - 145), both in the absence and in the presence of bombesin.

EXAMPLE 1

Boc-pMel-Gln-Trp-Ala-Val-Gly-OH (III)

Step 1 Boc-pMel-OH (I)

0.684 g (1.85 mmol) of H-pMel-OEt.HCl (F.Bergel and J.A. Stock (1954) J.Chem. Soc. 2409-2417) and 0.485 g (2.2 mmol) of $(Boc)_2O$ were dissolved in 40 ml of water and 8 ml of t-BuOH. The solution was adjusted to pH 10 with 1N NaOH, stirred for 15 min, then 40 ml of water and 110 ml of MeOH were added, and the pH brought to 13.5 with 1 NaOH. The reaction mixture was stirred for 1 hr a room temperature, then brought to pH 8.5 with 1N HCl and concentrated in vacuo. The aqueous solution was washed with n-hexane (4x30 ml),then cooled to -5°C, acidified to pH 2 with 1N HCl under stirring, and extracted with cooled AcOEt (4 x 30 ml). The organic layers were pooled, washed to neutrality with saturated solution of NaCl,

dried over anhydrous Na_2SO_4, filtered and evaporated in vacuo. The residue was dissolved in a mixture of CH_2Cl_2/AcOH 99/1 and purified by flash chromatography on silica gel eluting with the same solvent mixture. 0.7 g (77.8% yield) of product I were obtained as an oil: R_{fA} 0.70.

Step 2 Boc-pMel-Gln-Trp-Ala-Val-Gly-OBzl (II)

0.6 g of Boc-pMel-OH (I) (1.48 mmol) were dissolved in 10 ml of anydrous THF. The solution was cooled to -20°C, and 0.16 ml (1.48 mmol) of NMM and 0.15 ml (1.48 mmol) of ECC were successively added. After stirring at this temperature for 2 min, a cold solution of 1.02 g (1.48 mmol) of H-Gln-Trp-Ala-Val-Gly-OBzl . HCl (our UK patent application n°8808768.9), and 0.16 ml (1.48 mmol) of NMM in 10 ml of anhydrous DMF, was added. The reaction mixture was stirred for 2 h at -10° to -15°C, then filtered and evaporated in vacuo.

The residue was dissolved in 20 ml of DMF and poured dropwise into 40 ml of a 10% solution of citric acid at 5°C. The mixture was stirred for 1 h at a temperature below 10°C, then filtered and washed with water to neutrality. 1.4 g (91.5% yield) of product II were obtained: R_{fB} 0.48

Step 3 Boc-pMel-Gln-Trp-Ala-Val-Gly-OH (III)

0.44 g of 10% Pd/C and 24 ml of a pre-warmed solution made from 1.2 ml of HCOOH, 3.3 ml of NMM and 100 ml of MeOH, were added to a

solution of 1.2 g (1.16 mmol) of Boc-pMel-Gln-Trp-Ala-Val-Gly-OBzl (II) in 24 ml of anhydrous DMF. The reaction mixture was stirred for 15 min at 40°C , then cooled to room temperature, filtered and evaporated in vacuo. The residue was dissolved in DMF and precipitated with AcOEt, giving 1.1 g of crude product. This was purified by counter current distribution in the solvent system: water/DMF/n-BuOH/AcOEt = 40/3/20/80. Fractions containing the pure product were pooled and evaporated in vacuo. The residue was ground in DMF, MeOH and AcOEt, giving 0.680 g (63% yield) of product III: R_{fD} 0.54; RT_A 8.4; AA ratios; Glu 0.93 (1), Gly 0.99 (1), Ala 0.99 (1), Val 1 (Trp and pMel n.d.).

Example 2

Boc-pMel-Gln-Trp-Ala-Val-Gly-His(Dnp)-Leu-Met-NH_2(IV)

To a solution of 0.330 g (0.35 mmol) of Boc-pMel-Gln-Trp-Ala-Val-Gly-OH (III) in 3 ml of anhydrous DMF, 0.053 g (0.39 mmol) of anhydrous HOBt, 0.081 g (0.39 mmol) of DCC, 0.235 g (0.39 mmol) of H-His(Dnp)-Leu-Met-NH_2 . HCl (F.Angelucci and R. de Castiglione (1975) Experientia, 507-508) and 0.043 ml (0.39 mmol) of NMM were successively added. The reaction mixture was stirred at 0°C for 1 h and at room temperature for 30 h, then it was filtered and evaporated in vacuo. The residue was dissolved in 3 ml of anhydrous DMF, poured dropwise into 30 ml of an aqueous solution of 6 g NaCl and 3 g citric acid at a temperature below 10°C. After stirring for 1 h at a temperature < 10°C, the suspension was filtered and the

product was washed to neutrality. The crude material was evaporated twice from 10 ml of anhydrous DMF, then dissolved in 3 ml of anhydrous DMF and poured dropwise into 30 ml of an aqueous solution of 1.5 g $NaHCO_3$ and 6g NaCl at a temperature below 10°C. The mixture was stirred for 1 h, then filtered and washed with water to neutrality, giving 0.5 g (95.6% yield) of product IV: R_{fD} = 0.84; RT_A 21.2; AA ratios: Trp 0.97 (1), Glu 1.00 (1), Gly 1, Ala 1.00 (1), Val 1.00 (1), Met 1.10 (1), Leu 1.04 (1) (pMel and His(Dnp) n.d.).

Example 3

H-pMel-Gln-Trp-Ala-Val-Gly-His(Dnp)-Leu-Met-NH_2 . HCl (V)

0.100 g (0.067 mmol) of Boc-pMel-Gln-Trp-Ala-Val-Gly-His(Dnp)-Leu-Met-NH_2 (IV) were made to react with 1 ml of 1.33N HCl/AcOH containing, 0.2 ml of 2-mercaptoethanol and 0.1 ml of anisole. The reaction mixture was stirred for 30 min at room temperature, then evaporated in vacuo. The residue was ground with Et_2O, giving 0.080 g (83.5% yield) of product V: R_{fC} = 0.57; RT_A 9.3; AA ratios: Glu 0.99 (1), Gly 0.98 (1), Ala 1.04 (1), Val 1.10 (1), Met 0.82 (1), Leu 0.81 (1) (Trp, pMel and His(Dnp) n.d.).

Example 4

Boc-pMel-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2 (VI)

0.4 g (0.27 mmol) of Boc-pMel-Gln-Trp-Ala-Val-Gly-His(Dnp)-Leu-Met-NH_2 (IV) were dissolved in 400 ml of anhydrous DMF, then 5.36 ml of 0.1 M KH_2PO_4 (brought to pH 8.1 with 1N KOH) and 20 ml of 2-mercaptoethanol were added. The reaction mixture was stirred for 2 h at room temperature, then concentrated in vacuo. The residue was purified by counter-current distribution in the solvent system: water/n-BuOH/AcOH = 40/35/1. Fractions containing the pure product were pooled and evaporated in vacuo, giving 0.35 g (theorical yield) of product VI: R_{fc} 0.63; R_{fD} 0.82; RT_A 14.2; AA ratios: Glu 1, Gly 1.00 (1); Ala 1.04 (1), Val 1.04 (1); Met 0.94 (1), Leu 1.02 (1), His 0.95 (1), Trp 0.88 (1) (pMel n.d.).

Example 5

H-pMel-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2 . 2 HCl (VII)

0.1 g (0.075 mmol) of Boc-pMel-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2 (VI) were deblocked as described in example 3, obtaining 0.089 g (91% yield) of product VII: R_{fc} 0.45; RT_B 11.3; AA ratios: Glu 1.06 (1), Gly 1.00 (1), Ala 0.99 (1), Val 1, Met 0.94 (1), Leu 0.96 (1), His 0.94 (1) (Trp and pMel n.d.).

Example 6

Boc-pMel-Gln-Trp-Ala-Val-Gly-phe-Leu-Met-NH_2 (VIII)

0.15 g (0.16 mmol) of Boc-pMel-Gln-Trp-Ala-Val-Gly-OH (III) were dissolved in 12 ml of anhydrous DMF, and 0.023 g (0.169 mmol) of anydrous HOBt were added. To the solution, cooled at 0°C, 0.041 g (0.177 mmol) of DCC, 0.085 g (0.192 mmol) of H-phe-Leu-Met-NH_2 . HCl (our UK Patent Appl. n° 8808768.9, example 1 - step 14) and 0.022 ml of NMM (0.192 mmol) were added successively.
After stirring for 15 min at 0°C, 0.002 g (0.016 mmol) of DMAP were added. The reaction mixture was stirred for 1 h at 0°C and overnight at room temperature, then filtered and evaporated in vacuo. The residue was dissolved in 3 ml of anhydrous DMF and poured dropwise into 30 ml of an aqueous solution of 3 g citric acid and 6 g NaCl, at a temperature below 10°C. After stirring for 1 h, the solid was filtered and washed with water to neutrality. The product was then dissolved in 10 ml of anhydrous DMF and evaporated in vacuo. The residue was ground with Et_2O, giving 0.190 g (88.8% yield) of crude compound VIII. A sample was purified by reverse phase semi-preparative HPLC using a linear gradient system of 0.05% TFA (A) and 0.05% TFA/CH_3CN= 3/7 (B), from 70% to 90% B: R_{fc} 0.85; RT_A 18.4; AA ratios: Glu 0.90 (1); Gly 1.14 (1), Ala 1.02 (1), Val 0.99 (1); Met 0.94 (1), Leu 1.08 (1), phe 0.96 (1), Trp 0.96 (1) (pMel n.d.).

Example 7

Boc-mMel-Gln-Trp-Ala-Val-Gly-OH (IX)

The title compound was obtained as described in Example 1, starting from Boc-mMel-OH, obtained in turn from H-mMel-OH (H.F. Gram et al. (1963) J. Med. Chem., 6, 85-87): R_{f_D} 0.56; RT_A 8.5; AA ratios: Glu 0.93 (1), Gly 0.95 (1), Ala 0.95 (1), Val 1 (Trp and mMel n.d.).

Example 8

Boc-mMel-Leu-Gly-Thr-Gln-Trp-Ala-Val-Gly-Leu-Met-NH2 (XIV)

Step 1 Boc-Leu-Gly-OBzl (X)

2.33 ml (20.7 mmol) of NMM and 2.91 ml (20.7 mml) of isobuthyl-chlorocarbonate were successively added to a solution, cooled at -25°C, of 4.8 g (20.7 mml) of Boc-Leu-OH in 70 ml of anhydrous THF. After stirring the reaction mixture for 3 min at ca. -12°C, a cold solution of 6.98 g (20.7 mmol) of H-Gly-OBzl.TsOH and 2.33 ml (20.7 mmol) of NMM in 50 ml of anhydrous DMF was added. The reaction mixture was stirred for 45 min at ca. -12°C and for 90 min at 0°C, then filtered and evaporated in vacuo. The residue was dissolved in AcOEt and washed several times successively with a 10% aqueous solution of citric acid, brine, a 5% solution of $NaHCO_3$ and brine again. The organic layer was dried over anhydrous Na_2SO_4 and the solvent removed in vacuo, obtaining 7.8 g (100 % yield) of compound X as an oil: R_{f_B} 0.83; RT_A 13.6.

Step 2 H-Leu-Gly-OBzl . HCl (XI)

7.4 g (19.6 mmol) of Boc-Leu-Gly-OBzl (X) was deblocked as described in Example 3. The oily residue was ground several times with petroleun ether, obtaining 3.94 g (63.8% yield) of compound XI: R_{fc} 0.59.

Step 3 Boc-mMel-Leu-Gly-OBzl (XII)

5.7 g (12.51 mmol) of Boc-mMel-OH and 3.94 g (12.51 mmol) of H-Leu-Gly-OBzl . HCl (XI) were condensed as described in Example 1 - step 2. The residue was dissolved in AcOEt and washed several times successively with a 10% citric acid solution, brine, a 5% $NaHCO_3$ solution and brine again. The organic layer was dried over anhydrous Na_2SO_4 and the solvent removed in vacuo. The residue was purified by flash chromatography on silica gel, eluting with AcOEt/Et_2O=1/7. 6.25 g (75% yield) of product XII were obtained as a foam : R_{ff} 0.80.

Step 4 Boc-mMel-Leu-Gly-OH (XIII)

6.0 g (9.0 mmol) of Boc-mMel-Leu-Gly-OBzl (XII) were treated as described in Example 1 - step 3. The residue was ground with AcOEt/Et_2O/petroleum ether, giving 4.3 g (83% yield) of compound XIII: R_{ff} 0.43.

Step 5 Boc-mMel-Leu-Gly-Thr-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2 (XIV)

0.092 g (0.16 mmol) of Boc-mMel-Leu-Gly-OH (XIII) and 0.105 g (0.16 mmol) of H-Thr-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2.HCl (our UK Patent Appl. n° 8808768.9, Example 4) were condensed as described in Example 2. After evaporation of the solvent, the residue was dissolved in 10 ml of DMF, poured dropwise in 100 ml of a 10% solution of citric acid, stirred for 15 min, then filtered and washed to neutrality. The crude product was dissolved in 30 ml of DMF and evaporated in vacuo. The residue was ground with DMF/MeOH/ AcOEt/Et_2O, giving 0.17 g (72.7% yield) of crude compound XIV. A sample was purified by semi-preparative HPLC as described in Example 6: R_{fc} 0.84; RT_A 16.5; AA ratios: Thr 0.94 (1), Glu 1.06 (1), Gly 2.12 (2), Ala 0.94 (1), Val 0.94 (1), Met 1.00 (1), Leu 2, Trp 0.87 (1) (mMel n.d.).

In an analogous manner the following peptides have also been synthetized.

XV H-pMel-Gln-Trp-Ala-Val-Gly-phe-Leu-Met-NH_2 . HCl

R_{fc} 0.72; RT_A 9.6.

XVI Boc-mMel-Gln-Trp-Ala-Val-Gly-His(Dnp)-Leu-Met-NH_2

R_{fD} 0.86; RT_A 21.5; AA ratios: Glu 1, Gly 0.99 (1), Ala 1.03 (1), Val 0.99 (1), Met 1.03 (1), Leu 1.04 (1), Trp 1.05 (1) (mMel and His(Dnp) n.d.)

XVII H-mMel-Gln-Trp-Ala-Val-Gly-His(Dnp)-Leu-Met-NH_2 . HCl
R_{fc} 0.74; RT_A 9.4; AA ratios: Glu 0.99 (1), Gly 1.04 (1), Ala 1.02 (1), Val 1, Met 0.96 (1), Leu 0.96 (1) (His(Dnp) Trp and mMel n.d.)

XVIII Boc-mMel-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2.CF_3COOH
R_{fc} 0.55; R_{fD} 0.80; RT_A 14.4; AA ratios: Glu 1, Gly 0.99 (1), Ala 0.99 (1), Val 1.06 (1), Met 1.05 (1), Leu 0.97 (1), His 0.90 (1), Trp 1.03 (1) (mMel n.d.)

XIX H-mMel-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2 . 2 HCl
R_{fc} 0.44; RT_B 11.3; AA ratios: Glu 1.00 (1), Gly 1.00 (1), Ala 1.05 (1), Val 1, Met 0.91 (1), Leu 0.89 (1), His 0.99 (1), (mMel and Trp n.d.)

XX Boc-mMel-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2
R_{fc} 0.74; RT_A 15.4; AA ratios: Glu 1.03 (1), Gly 1, Ala 1.04 (1), Val 0.96 (1), Met 0.93 (1), Leu 1.02 (1), Trp 0.94 (1) (mMel n.d.)

XXI H-mMel-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2 . HCl
R_{fc} 0.65; RT_A 5.7

XXII H-mMel-Leu-Gly-Thr-Gln-Trp-Ala-Val-Gly-Leu-NH_2 .HCl
R_{fc} 0.52; RT_A 6.6

XXIII H-pMel-Leu-Gly-Thr-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2 .HCl

XXIV H-pMel-Gln-Trp-Ala-Val-ala-His-Leu-Met-NH_2 .2HCl

XXV H-pMel-Asn-Gln-Trp-Ala-Val-Gly-Leu-Nle-NH_2 .HCl

XXVI H-pMel-Asn-Gln-Trp-Ala-Val-Gly-Leu-NH$(CH_2)_4CH_3$ .HCl

XXVII H-pMel-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2 .HCl

XXVIII H-pMel-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-Leu-Met -NH_2 .2HCl

XXIX H-pMel-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-ala-Leu-Nle -NH_2 .HCl

XXX H-pMel-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-ala-Leu-NH $(CH_2)_2C_6H_5$ .HCl

XXXI H-Glp-Arg-Leu-Gly-pMel-Asn-Gln-Trp-Ala-Val-Gly-Leu-Nle -NH_2 .HCl

XXXII H-Leu-Gly-pMel-Gln-Trp-Ala-Val-Gly-phe-Leu-Nle-NH_2 .HCl

XXXIII H-Leu-Gly-pMel-Gln-Trp-Ala-Val-Gly-ala-Leu-Nle-NH_2 .HCl

XXXIV H-pMel-Asn-Gln-Trp-Ala-Val-Gly-Leu-Leu-NH-NH_2 .HCl

XXXV Boc-pMel-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2
Rf_C 0.86;RT_A 15.3; AA ratios: Glu 1.02(1), Gly 1.07(1), Ala 1.10(1), Val 1, Met 0.92(1), Leu 0.98(1)(Trp and pMel n.d.)

XXXVI H-pMel-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2.HCl
Rf_C 0.57; RT_B 16.8; AA ratios:Glu 1.08(1), Gly 1.01(1), Ala 0.98(1), Val 1, Met 0.90(1), Leu 0.94(1)(Trp and pMel n.d.)

XXXVII Boc-Lys(Boc)-Gly-pMel-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2
Rf_C 0.73; RT_A 18.7; AA ratios: Glu 1.07(1), Gly 2.02(2) Ala 1.18(1), Val 1, Met 0.88(1), Leu 0.95(1), Lys 1.08(1) (Trp and pMel n.d.)

XXXVIII H-Lys-Gly-pMel-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2. $2CF_3COOH$
Rf_E 0.71; RT_B 15.4; AA ratios: Glu 0.99(1), Gly 2.02(2), Ala 1.00(1), Val 1, Met 0.88(1); Leu 0.91(1), Lys 1.15(1), Trp 0.91(1)(pMel n.d.)

XXXIX Ac-Lys(Boc)-Gly-pMel-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2
Rf_C 0.77; RT_A 11.9; AA ratios: Glu 1.05(1), Gly 1.97(2), Ala 0.98(1), Val 1, Met 0.89(1), Leu 0.92(1), Lys 0.92(1), Trp 0.88(1)(pMel n.d.)

XL Ac-Lys-Gly-pMel-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2.CF_3COOH
Rf_D 0.78; RT_B 16.2, AA ratios: Glu 1; Gly 2.11(2); Ala 0.99(1); Val 0.89(1); Met 0.91(1); Leu 0.92(1); Lys 1.10(1)(Trp and pMel n.d.)

XLI Boc-pMel-Leu-Gly-Thr-Gln-Trp-Ala-Val-His(Dnp)-Leu-
-$NH(CH_2)_4CH_3$
Rf_C 0.88; RT_A 24.2: AA ratios: Thr 1.04(1), Glu 0.96(1), Gly 2, Ala 1.03(1), Val 0.95(1), Leu 1.92(2) (His(Dnp), Trp and pMel n.d.)

XLII H-pMel-Leu-Gly-Thr-Gln-Trp-Ala-Val-His(Dnp)- Leu -
$NH(CH_2)_4CH_3$.HCl
Rf_C 0.50: RT_A 18.7: AA ratios: Thr 0.92(1). Glu 0.97(1), Gly 2.04(2), Ala 1.04(1), Val 1, Leu 1.88(2) (His(Dnp), Trp and pMel n.d.)

XLIII Boc-pMel-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-ala -
Leu-Nle-NH_2.CF_3COOH
RT_A 18.4

XLIV H-pMel-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-ala-Leu-Nle-
-NH_2.$2CF_3COOH$

TABLE 1

BINDING AFFINITY OF BOMBESIN ALKYLATING ANALOGUES ON MOUSE SWISS 3T3 FIBROBLASTS

COMPOUND	ID_{50} (nM)
III	12,000± 400
IX	8,200±680
VII	60±20
V	680±150
XIX	95±8
XVII	148±27
VI	48±2
IV	1,170±400
XVIII	40±12
XVI	390±160
XX	60.1±3.3
XIV	445±60

Reference peptides:

BBS	12.6±3.8
Spantide	11,100
[pro²]Spantide	14,000
[$Leu^{13}\Psi(CH_2\text{-}NH)Leu^{14}$]BBS	214±30

TABLE 2

[H³]THYMIDINE INCORPORATION IN MOUSE SWISS 3T3 FIBROBLASTS

COMPOUND	FOLD INCREASE OVER BASAL VALUE				% INHIBITION IN THE PRESENCE OF 25nM BBS			
					A		B	
	5nM	50nM	500nM	5000nM	500nM	5000nM	500nM	5000nM
III	-	-	1.2	1.3	0	64±10	27±14	39±7
IX	-	-	1	1	0	57±13	17±4	22±3
VII	2.1	4.7	4.3	4.8	6 ± 2	17 ± 4	57 ± 14	61 ± 9
V	1	1	1.4	1.8	26 ± 8	44 ± 12	87 ± 9	83 ± 6
XIX	1	4.1	4.3	4.4	19 ± 7	9 ± 5	54 ± 1	62 ± 6
XVII	1	2.9	4.2	3.9	6 ± 3	20 ± 7	58 ± 13	62 ± 1
VI	4.1	8.0	7.0	6.6	3 ± 2	20 ±3	21 ± 3	34 ± 5
IV	1	1	1.7	2.3	59 ± 3	67 ± 3	81	73
XVIII	5.4	7.0	7.3	5.4	4 ± 2	3 ± 1	3 ± 2	14 ± 3
XVI	1.2	1.6	3.1	3.9	17 ± 2	41 ± 1	47 ± 10	37 ± 7
VIII	-	1.1	1.2	1.2	28 ± 6	37 ± 4	56 ± 3	77 ± 11
XX	-	1.0	1.5	1.2	39 ± 1	68 ± 8	0	35 ± 3
XIV	-	1.2	1.3	1.2	6 ± 2	14 ± 2	0	32 ± 8
Reference peptides:								
BBS	3.0 ± 1							
[Leu$^{13}\Psi$(CH$_2$-NH)Leu^{14}]BBS			1.0	1.0	29 ± 10	56 ± 4	0	0

A= analogues are given in combination with BBS

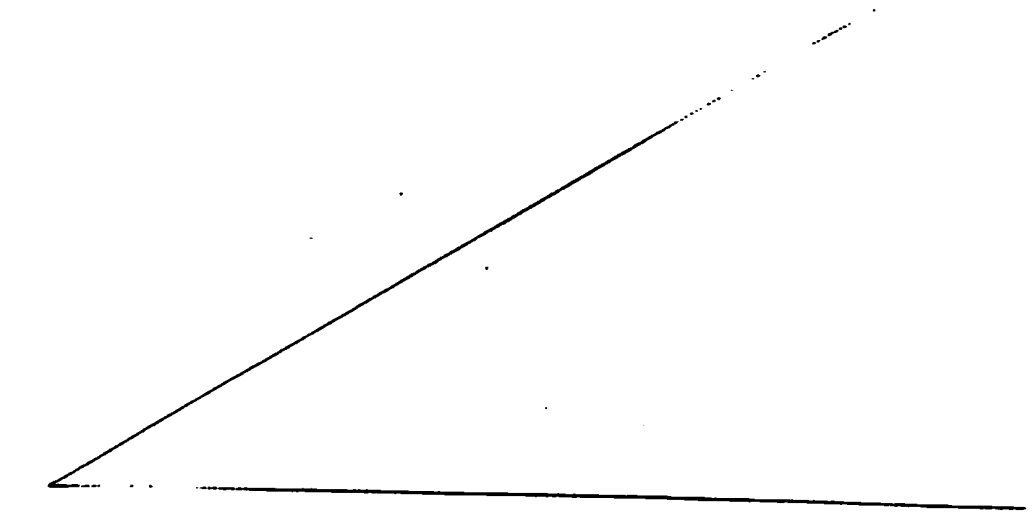
B= cells are pre-treated with analogues, washed, left at 37°C for 24 h and then challenged with BBS

TABLE 3

PHOSPHORYLATION OF THE p115 PROTEIN ASSOCIATED WITH THE BOMBESIN RECEPTOR

COMPOUND	MINIMAL ACTIVE DOSE (nM)
III	> 10000
VII	1
V	100
XIX	4
XVII	4
VI	1
IV	50
XVIII	10
XVI	40
XX	> 500
XIV	> 1000
Reference peptides:	
BBS	3
Spantide	> 10000

From the above tables, it is evident that when the alkylating moiety was introduced into an agonist structure (compounds VI, VII, XVIII, XIX) the resulting alkylating analogs increased thymidine incorporation when given alone, and were weak antagonists when given together with BBS but potent antagonists when given 24 hrs before the BBS addition. When the alkylating moiety was introduced into BBS analogues which are "per se" inactive (compounds III and IV) or weak antagonists (compounds IV, V, VIII, XIV, XVI, XVII and XX) the resulting alkylating compounds did not increase incorporation of thymidine and they usually behaved as potent antagonists either when given contemporaneously with BBS or when given 24 hrs after BBS treatment.



CLAIMS:

1. A peptide of the formula I:

A-B-C-D-Gln-Trp-Ala-Val-X-Y-T-W I

wherein

either

A represents a hydrogen atom, a Boc or an acetyl group,
one of B and C represents a pMel or mMel residue,
and
the other of B and C represents a valence bond or a Gly, Leu-Gly, E -Leu-Gly or Gln- E -Leu--Gly ,E,E-Gly residue with E = Arg (A), arg (A), Lys (A), lys (A), Orn (A) and orn (A), or
A represents a hydrogen atom,
B represents a Glp-Arg-Leu-Gly residue, and
C represents a pMel or mMel residue,

and

D represents a valence bond or an Asn or Thr residue,
X represents a Gly or ala residue,
Y represents a valence bond or a His(R_1), his(R_1), Phe, phe, Ser, ser, Ala or ala residue,
T represents a valence bond or a Leu, leu, Phe or phe residue,
W represents an OH, amino, pentylamino or phenethylamino group or Met-R_2, Leu-R_2, Ile-R_2 or Nle-R_2 residue,

R_1 represents a hydrogen atom or a Tos, Dnp or Bzl group, and
R_2 represents an amino, hydroxy, methoxy or hydrazino group;

or a pharmaceutically acceptable salt of such a peptide.

2. Any one of the following peptides:

Boc-pMel-Gln-Trp-Ala-Val-Gly-OH

Boc-mMel-Gln-Trp-Ala-Val-Gly-OH

Boc-pMel-Gln-Trp-Ala-Val-Gly-His(Dnp)-Leu-Met-NH_2

H-pMel-Gln-Trp-Ala-Val-Gly-His(Dnp)-Leu-Met-NH_2

Boc-pMel-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2

H-pMel-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2

Boc-pMel-Gln-Trp-Ala-Val-Gly-phe-Leu-Met-NH_2

Boc-mMel-Leu-Gly-Thr-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2

H-pMel-Gln-Trp-Ala-Val-Gly-phe-Leu-Met-NH_2

Boc-mMel-Gln-Trp-Ala-Val-Gly-His(Dnp)-Leu-Met-NH_2

H-mMel-Gln-Trp-Ala-Val-Gly-His(Dnp)-Leu-Met-NH_2

Boc-mMel-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2

H-mMel-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2

Boc-mMel-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2

H-mMel-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2

H-mMel-Leu-Gly-Thr-Gln-Trp-Ala-Val-Gly-Leu-NH_2

H-pMel-Leu-Gly-Thr-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2

H-pMel-Gln-Trp-Ala-Val-ala-His-Leu-Met-NH_2

H-pMel-Asn-Gln-Trp-Ala-Val-Gly-Leu-Nle-NH_2

H-pMel-Asn-Gln-Trp-Ala-Val-Gly-Leu-$NH(CH_2)_4CH_3$

H-pMel-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2

H-pMel-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2

H-pMel-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-ala-Leu-Nle-NH_2

H-pMel-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-ala-Leu-$NH(CH_2)_2C_6H_5$

H-Glp-Arg-Leu-Gly-pMel-Asn-Gln-Trp-Ala-Val-Gly-Leu-Nle-NH_2

H-Leu-Gly-pMel-Gln-Trp-Ala-Val-Gly-phe-Leu-Nle-NH_2

H-Leu-Gly-pMel-Gln-Trp-Ala-Val-Gly-ala-Leu-Nle-NH_2

H-pMel-Asn-Gln-Trp-Ala-Val-Gly-Leu-Leu-NH-NH_2

Boc-pMel-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2

H-pMel-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2

Boc-Lys(Boc)-Gly-pMel-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2

H-Lys-Gly-pMel-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2

Ac-Lys(Boc)-Gly-pMel-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2

Ac-Lys-Gly-pMel-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2

Boc-pMel-Leu-Gly-Thr-Gln-Trp-Ala-Val-His(Dnp)-Leu-$NH(CH_2)_4CH_3$

H-pMel-Leu-Gly-Thr-Gln-Trp-Ala-Val-His(Dnp)-Leu-$NH(CH_2)_4CH_3$

Boc-pMel-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-ala-Leu-Nle-NH_2 or

H-pMel-Gln-Arg-Leu-Gly-Asn-Glu-Trp-Ala-Val-ala-Leu-Nle-NH_2

3. A pharmaceutical composition comprising a peptide according to claim 1 or claim 2 or a pharmaceutically acceptable salt of such a peptide in admixture with a pharmaceutically acceptable diluent or carrier.

4. A process for the preparation of a peptide according to claim 1, the process comprising condensing amino acids and/or amino acid derivatives in the desired sequence and/or peptide fragments containing these amino acids or their derivatives in the desired sequence to give the desired peptide, either an end carboxylic acid group or an end amino group being activated for the peptide linkage and the remaining groups being protected, and optionally deprotecting the resultant peptide and/or converting the resultant peptide into a pharmaceutically acceptable salt thereof.

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 89/00842

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: C 07 K 7/06, C 07 K 7/08, A 61 K 37/02

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	C 07 K, A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	FR, A, 2100975 (SOCIETA FARMACEUTICI ITALIA) 24 March 1972, see the whole document --	1,2,3
Y	Chemical Abstracts, vol. 110, 1989 (Columbus, Ohio, US), J. Hepp et al.: "Melphalan analog of the dynorphin-(1-7)-heptapeptide", page 761, abstract no. 95784k, & F.E.C.S. Int. Conf. Chem. Biotechnol. Biol. Act. Nat. Prod., (Proc.), 3rd 1985, see abstract --	1,3
Y	Chemical Abstracts, vol. 109, 1988, (Columbus, Ohio, US), J. Hepp et al.: "Synthesis of novel opioid peptides possessing an alkylating effect and investigation of their coupling to receptors", page 86, abstract no. 48561e, & Kem. Kozl. 1986, 65(1), 34-43, see abstract --	1,3

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* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
3rd October 1989	3. 11. 89
International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	T.K. WILLIS

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

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A	US, A, 4331661 (MARKI) 25 May 1982, see the whole document	1,3
A	Chemical Abstracts, vol. 109, 1988, (Columbus, Ohio, US), Ye.N. Shkodinskaya et al.: "Alkylating derivatives of 2-amino-2-deoxysaccharides and 1-methylamino-1-deoxypolyols (synthesis and experimental study)", page 728, abstract no. 55184n, Vopr. Onkol. 1987, 33(11), 48-53, see abstract	1,3
P,A	Chemical Abstracts, vol. 108, 1988, (Columbus, Ohio, US), N.D. Lagova et al.: "The use of peptide hormone melanostatin and its analogs for synthesis of new antitumor compounds", page 101, abstract no. 180442a, & Vopr. Onko. 1988, 34(1), 73-9, see abstract	1,3
A	US, A, 4207311 (BROWN) 10 June 1980, see the whole document	1,3

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. EP 8900842 SA 29869

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 25/10/89 The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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		DE-A,B,C 2137971	03-02-72
		GB-A- 1309728	14-03-73
		US-A- 3792032	12-02-74
US-A- 4331661	25-05-82	None	
US-A- 4207311	10-06-80	None	